**Supplementary Material**

**Quantitative RT-PCR**

2x105 CRC cells were seeded in complete culture medium in 6-well plates. Transfected cells were incubated under their normal growth conditions (37°C, 5% CO2) and the effect of miR-188-3p manipulation on changes in gene expression levels of putative target genes was measured by RT-qPCR after 48 hours. Therefore, 1 μg of total RNA was reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer´s protocol. RT-qPCR was carried out in technical duplicates of biological triplicates using specific primer pairs. Primer sequences for NLK, CTNAA2, MLLT4, GAPDH and B2M are:

NLK\_fw GGGGTCCTCATAAACAGCCAT

NLK\_rev ACCAACATCCTGCAAAGGAGA

CTNNA2\_fw CTGATCAGCTGGACAGTGCC

CTNNA2\_rev AGGATGCTTTCACCGTGAGG

MLLT4\_fw AAGCTGGCCGACATCATCC

MLLT4\_rev AACTCCAAATCCTCGGTCGG

GAPDH\_fw AAGGTCGGAGTCAACGGATTT

GAPDH\_rev ACCAGAGTTAAA AGCAGCCCTG

B2M\_fw TGCTGTCTCCATGTTTGATGTATCT

B2M\_rev TCTCTGCTCCCCACCTCTAAGT

. The RT-qPCR reactions was carried out on a LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany) using the QuantiTect SYBR Green PCR Kit (Qiagen) according to the manufacturer’s protocol. The arithmetic mean of the housekeeping genes GAPDH and B2M were used for normalization and relative gene expression levels were calculated using the 2−ΔΔCt method.

**Western blot**

For measuring the effects of miR-188-3p transfection on protein expression levels, total protein of transiently transfected HCT116 and HRT-18 cells were extracted with radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 1% Triton, 0.1% SDS, 0.1% sodium deoxycholate and 1% Nonidet P40). 20 μg of total cellular protein were resuspended in laemmli buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromphenol blue and 0.125 M Tris HCl, pH approx. 6.8) and heated at 65°C for 10 minutes. Proteins were separated by a 4–15% Mini-PROTEAN® TGX™ Precast Gel (Biorad, Hercules, CA) and transferred onto a nitrocellulose membrane (Applichem, St. Louis, MO). The membrane was blocked for 1 hour with 3% nonfat dry milk in Tris buffered Saline/0.1% Tween-20. Primary antibodies specific for β-Actin (Sigma, Seelze, Germany, Cat.No. A5441, clone AC-15, diluted 1:3000 in 1% nonfat dry milk in Tris buffered Saline/0.1% Tween-20) and MLLT4 (Cell Signaling, Danvers, MA, Cat.No. 6492, diluted 1:1000 in 1% nonfat dry milk in Tris buffered Saline/0.1% Tween-20) were used to detect the expression levels after using HRP-conjugated anti-mouse or anti-rabbit secondary antibodies, respectively (Dako, Glostrup, Denmark). Visualization was performed using an enhanced chemoluminescence detection system (Super Signal West Pico, Thermo Scientific, Rockford, IL). Relative quantification of protein expression was performed using the ImageJ (NIH, Bethesda, Maryland) software. Therefore, the band density of the protein of interest was measured and divided by the density of the loading control beta actin.

**Transient Transfection of miR-188-3p**

For functional studies a specific miR-188-3p mimetic (Syn-Hsa-mir-188-3p, Qiagen) and a respective negative control (miScript Negative Control, Qiagen) were commercially purchased. MiRNA transfection complexes were added according to the fast forward or reverse transfection protocol as recommended by the manufacturer (concentration of 50nM; HighPerfect Transfection Reagent, Qiagen).

**SiRNA Transfection**

CRC cells were transiently transfected with siRNA (50 nM) for MLLT4 mRNA using the fast forward transfection procedure according to the HiPerFect Transfection Reagent (Qiagen) protocol. A specific siRNA against MLLT4 mRNA (Hs\_MLLT4\_1, Qiagen) and, as a negative control, the All Star Negative Control (Qiagen) were used.

**Lentiviral Transduction**

HCT116 cells were seeded in 6-well plates 24 hours prior to viral infection and incubated overnight in complete growth medium. On the day of transfection, the medium was replaced with complete growth medium containing 8 μg/ml polybrene (Santa Cruz Biotechnology, Santa Cruz, CA) and 10 μl of ViralPlus Transduction Enhancer (ABM, Richmond, BC, Canada). Cells were infected by adding 10 μl of miR188-3p overexpression lentiviral particles (shMIMIC Human Lentiviral microRNA hsa-miR-188-3p, Dharmacon) or blank control lentiviral particles (ABM), respectively. Stably transfected HCT116 cells were selected with 0.5 μg/ml puromycin dihydrochloride (Gibco, Carlsbad, CA).

**Labelling of cells with luciferase gene for in vivo imaging technique**

For the purpose of in vivo imaging by using luciferase-labelled cells, CRC (HCT-116) cells were seeded in 12-well plates 24 hours prior to viral infection and incubated overnight in 2 ml of complete growth medium containing 10% FBS and 1% antibiotics. On the day of transfection the medium was removed and 2 ml of complete growth medium containing 8 µg/ml polybrene (Santa Cruz Biotechnology, Santa Cruz, CA) and 5 µl of ViralPlus Transduction Enhancer (ABM, Richmond, BC, Canada) were added. Then, cells were infected by adding a Lentiviral Dual Reporter Imaging Construct (CMV-RFP-T2A-Luciferase, Biocat. Heidelberg, Germany) and grown up for 2-3 weeks before the in vivo experiment was initiated.

**REMARK criteria in relation to our study (**[**1**](#_ENREF_1)**)**

**INTRODUCTION**

1. State the marker examined, the study objectives, and any pre-specified hypotheses.

*Done in the introduction section.*

**MATERIALS AND METHODS**

Patients

2. Describe the characteristics (e.g., disease stage or comorbidities) of the study patients, including their source and inclusion and exclusion criteria.

*Done in the Methods section and Table S1.*

3. Describe treatments received and how chosen (e.g., randomized or rule-based).

*Done in the Methods section.*

Specimen characteristics

4. Describe type of biological material used (including control samples) and methods of preservation and storage.

*For cohort 1 (TCGA data) this information is publicly available, for the cohort 2 the information is described in every detail in the methods section.*

Assay methods

5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.

*We specified the methods, the kits and the material as well as the blinding procedure of the experiments for the biomarker study in the material and methods section.*

Study design

6. State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.

*We stated the details about a retrospective design, a cohort of consecutive cases, no matching of cases and the period of time of the study in the methods section.*

7. Precisely define all clinical endpoints examined.

*Clinical endpoint was defined in the methods section.*

8. List all candidate variables initially examined or considered for inclusion in models.

*We list all variables in the methods and results section examined in this study.*

9. Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.

*As this was a retrospective cohort study of two available study populations, no sample size or power calculations were initially performed.*

Statistical analysis methods

10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.

*Done in the methods/statistical section.*

11. Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.

*We used log-transformed data for expression levels and the median as a cut off which is frequently used and not prone for subjective “cut off optimization” in some other studies.*

**RESULTS**

Data

12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.

*We described every detail in the text of the results section.*

13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.

*We described every detail in the text of the results section and Supplementary Table S1.*

Analysis and presentation

14. Show the relation of the marker to standard prognostic variables.

*We describe these details in text of the results section and Table S4/Table 1.*

15. Present univariate analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan–Meier plot is recommended.

*We are showing this data in Table S4 and Figures 2 as well as Figure S3.*

16. For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.

*We are showing this data in Table 1.*

17. Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.

*We are showing this data in Table 1, Table S4 and the text section.*

18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.

*We describe the check of Cox model assumption in the statistical methods section.*

**DISCUSSION**

19. Interpret the results in the context of the prespecified hypotheses and other relevant studies; include a discussion of limitations of the study.

*We address all these points in our discussion section including the limitations of our study.*

*20. Discuss implications for future research and clinical value.*

*We discuss all these points in our discussion section.*

*1. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). Journal of the National Cancer Institute. 2005;97:1180-4.*